© Comparative Cytogenetics, 2009 . Vol. 3, No. 2, P. 85-89. ISSN 1993-0771 (Print), ISSN 1993-078X (Online)

AFLP diversity between the Novosibirsk and Tomsk chromosome races of the common shrew (*Sorex araneus*)

A.V. Polyakov[1], V.B. Ilyashenko[2], S.S. Onischenko[2], V.V. Panov[3], P.M. Borodin[1]

[1]Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Prosp. akademika Lavrentieva, 10, Novosibirsk 630090, Russia. [2]Department of Zoology and Ecology Kemerovo State University, Krasnaya ul., 6, Kemerovo 650043, Russia. [3]Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, ul. Frunze, 11, Novosibirsk 630091, Russia.
E-mails: polyakov@bionet.nsc.ru, borodin@bionet.nsc.ru

Abstract. Genetic diversity between of the Novosibirsk and Tomsk chromosome races of the common shrew (*Sorex araneus*) was analyzed using 39 polymorphic AFLP (amplified fragments length polymorphism) markers. Exact and *F*-statistics tests for population differentiation demonstrated significant interracial difference in allele frequencies and significant subdivision between the races. The value of the genetic distance between the chromosome races observed in this study corresponds to that found between subspecies of mammals studied so far.

Key words: *Sorex araneus*, chromosome races, AFLP, interracial diversity.

Introduction

The common shrew *Sorex araneus* Linnaeus, 1758 inhabits a huge territory including the whole north part of Europe and Asia up to Baikal Lake (Corbet, 1978) and displays a remarkable chromosomal polymorphism determined by local fixation of various Robertsonian and whole-arm reciprocal translocations (Searle, Wójcik, 1998). The species area is subdivided into a mosaic of parapatric chromosome races that has been classified into four main karyotypic groups: the West European (WEKG), the East European (EEKG), the North European (NEKG) and the Siberian (SKG) (Searle, 1984; Wójcik, 1993; Searle, Wójcik, 1998; Polyakov et al., 2001). Whereas no clear morphological divergence has been found between races within a karyotypic group (Hausser, 1984; Searle, Thorpe, 1987; Hausser et al., 1991; Meyer, Searle, 1994), significant morphological differences have been detected between the shrews belonging to different karyotypic groups: WEKG and EEKG in Poland (Chętnicki et al., 1996) and EEKG and SKG in Russia (Pavlinov, 2004).

Novosibirsk and Tomsk chromosome races belong to different karyotypic groups - EEKG and SKG correspondingly (Searle, Wójcik, 1998; Polyakov et al., 2001). The Novosibirsk race contains diagnostic chromosomes *go*,*hn*,*ik*,*mp*,*qr*; the Tomsk race - *gk*,*hi*,*mn*,*o*,*p*,*q*,*r*. The Novosibirsk race is distributed in the lowlands, while the Tomsk race – in the highlands of the West Siberia (Král,

Radjabli, 1974; Aniskin, Volobouev, 1981; Polyakov et al., 1996, 2000, 2001, 2003). They come into contact on the intermediate landscape and form there a very narrow hybrid zone (Polyakov et al., 2002, 2003).

It was known before the use of karyological data that shrews of Siberian highlands differed morphologically from other Siberian and Uralian shrew samples (Dolgov, 1968, 1985).

Yudin (1989), combining these earlier observations and his own analysis with pioneering descriptions of Siberian chromosome races by Aniskin and Volobouev (1981), first assumed that the morphological distinction between populations of shrews could be connected with their karyotypic divergence. Emphasizing the pronounced morphological and karyotypic individuality of shrews from Altai Yudin (1989) considered them as a subspecies *Sorex araneus rypheus* in distinction to the nominal subspecies *Sorex araneus araneus*.

Subsequent investigations confirmed that namely the shrews of the Tomsk chromosome race were quite distinct morphologically from the shrews of neighboring Novosibirsk race (Polyakov et al., 2002) as well as from some other EEKG races (Okulova et al., 2007).

In this study we estimated genetic diversity between samples from populations of the Novosibirsk and the Tomsk chromosome races of the common shrew using polymorphic AFLP (amplified fragments length polymorphism) markers. This method can generate large numbers of molecular markers without any previous knowledge of the genetic constitution of the genotypes under investigation (Vos et al., 1995). Because of its universal applicability, reproducibility and a high power of discrimination this approach has been used successfully in numerous phylogenetic and ecological studies (see review of Bonin at al., 2007). This is the first time this method is used to analyze populations of the common shrew.

Material and Methods

Animals

The shrews were collected within the known territories of two chromosome races: Tomsk race (Agendarovo Vill., Kemerovo Distr. 54°45'N/87°01'E) and Novosibirsk race (Novosibirsk vic. 54°49'N/83°06'E). Karyotype of each individual was determined by G-banding of standard bone marrow chromosome preparations.

AFLP procedure

We followed Vos et al. (1995) in the AFLP procedure. It was based on the selective PCR of restriction fragments from a total digest of genomic DNA by *Eco*RI and *Mse*I endonucleases. Two combinations of three randomly chosen primers complementary to *Eco*RI and *Mse*I adapters with three extra-nucleotide each (E-ACT/M-CCG and E-ACT/M-CGT) were used for the analytic amplification. Amplified fragments were resolved on 6% denaturing polyacrylamide gels and silver stained (Creste et al., 2001). Gels were then dried and scanned. Electrophoretic bands were scored with Gel-Pro Analyzer system version 3.0.00.00

Data scoring and statistical analysis

Only precisely and evenly expressed electrophoretic bands were chosen for analysis. Length of visible fragments of DNA on gels ranged between 60 and 800 nucleotides, however the area of fragments longer than 500 nucleotides was not clear enough. For this reason they were excluded from the analysis. We regarded each band as a locus with two alternative alleles: present (1) or absent (0). The identification of 39 polymorphic bands (=loci) led to the construction of a 47 individuals x 39 loci data matrix, which was analyzed for diversity within and between races. The genetic data was analyzed using the Tools for Population Genetic analysis (TFPGA) program ver-

Table 1. Summary statistics of the sample sizes, number and % (in parentheses) of polymorphic loci and Nei's (1973) index of gene diversity for the Novosibirsk and the Tomsk chromosome races.

Race	Sample size	Number of polymorphic loci	Gene diversity
Novosibirsk	24	31(79)	0.30±0.18
Tomsk	23	34(87)	0.37±0.17

sion 1.3 (Miller, 1997) for dominant markers in diploid organism, assuming Hardy–Weinberg equilibrium. Both band-based and allele frequency-based approaches were applied to extract the statistical information from AFLP data (Bonin at al., 2007). Exact test for population differentiation (Raymond, Rousset, 1995) was applied to determine significant differences in the bands frequencies between races. Genetic distance D_{NL} (Nei, Li, 1979) was calculated from the bands frequencies using the phylogenetic package PHYLIP 3.6 (RESTDIST and NEIGHBOR subroutines) (Felsenstein, 2005).

Unbiased genetic distance D_N (Nei, 1978) and gene diversity (Nei, 1973) were calculated on the base of allele frequencies. Frequency of the null allele was calculated as a square root of the frequency of null homozygotes (Weir, 1990).

To evaluate the substructure of the studied samples Weir and Cockerham's (1984) methods of calculating Wright's *F*-statistics were applied to the data. Two-level hierarchy (within and between races) was expressed using the terminology of Weir (1990) where θ correspond to unbiased Wright's F_{ST}. For hierarchical data set two θ values were calculated: θ_P measured differentiation within races and θ_R estimated the differentiation between the races. Jackknifing and bootstrapping over loci (using 1000 replications) were generated to obtain variance estimates and confidence intervals.

Results

A summary of the genetic diversity estimated from the AFLP data is given in Table 1. Amount of polymorphic loci and the levels of gene diversity within two races were about similar with slight priority within the Tomsk race sample. Exact test however demonstrated the high significance of the difference in bands frequencies between the races: χ^2=189.6 (P< 0.001). Two-level hierarchy *F*- statistics also revealed the significant differences among the intra and interracial substructures. Bootstrapping (1000 replications) confidence intervals of within and between races jackknifing variances θ estimates (θ_P=0.2237 ± 0.0366 and θ_R=0.0315 ± 0.0441) did not overlap at the 95% confidence level.

Two estimators of genetic distance between races gave the cognate data: D_N = 0.0713 and D_{NL} = 0.0983.

Discussion

Summarizing the series of observations Nei (1975) designed the scale of relatedness between the genetic distance and taxonomic categories. By this scale the genetic distance between local populations of a species ranges from 0 to 0.02 and from 0.02 to 0.2 between subspecies. Genetic differentiation between the Novosibirsk and Tomsk races, estimated by D_N and D_{NL} was higher than that between local populations. Nearly the same values of genetic distance were published recently for four sibling species of the blind mole rat *Spalax* ehrenbergi: *S. galili*, *S. golani*, *S. carmeli*, and *S. judaei* (Polyakov et al., 2007). The latter result was also obtained with the use of AFLP method and is thus completely compatible with our data. Like the shrew chromosome races Novosibirsk and Tomsk these

four species are distinguished by chromosome complements and form parapatric areas (Nevo et al., 2001).

Together with substantial genetic distance our analysis revealed statistically significant difference between the studied races in contents of their DNA polymorphism (Exact test and *F*- statistics). Thus, the whole set of existing knowledge about the Tomsk chromosome race (morphological, karyotypic and molecular specificity) indicate its taxonomical distinctiveness. This can be considered as evidence in favor of a subspecies status of the Tomsk chromosome race and its name *Sorex araneus rypheus* suggested by Yudin (1989). The shrews of other chromosome races of the Siberian Karyotypic Group may also belong to this subspecies. Analysis of Inter-SINE–PCR markers in a sample of shrews inhabiting at the right bank of the Yenisei river and apparently belonging to other than Tomsk chromosome race demonstrated a close clustering with the Tomsk race and was quite distinct from the races of the East European Karyotypic group (Bannikova et al., 2003). However, further studies and additional materials from Siberian populations are necessary to estimate genetic diversity within and between karyotypic groups and to test this suggestion.

Acknowledgements

This work was supported by research grants from INTAS, RFBR and the Program for collaborative researches of Siberian and Far Eastern Branches of the Russian Academy of Sciences.

References

Aniskin V.M., Volobouev V.T. 1981. Chromosome polymorphisms in Siberian populations of shrews of *araneus-arcticus* complex (Insectivora, Soricidae). Three chromosome forms of the common shrew *Sorex araneus* L. // *Genetika* (Moscow). 17: 1784-1791. (In Russian with English summary).

Bannikova A.A., Bulatova N.S., Krysanov E.Y., Kramerov D.A. 2003. DNA polymorphism within *Sorex araneus* and two congeneric species as inferred from inter-SINE-PCR // *Mammalia*. 67(2): 263-274.

Bonin A., Ehrich D., Manel S. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists // *Mol. Ecol.* 16: 3737-3758.

Chętnicki W., Fedyk S., Banaszek A., Szałaj K., Ratkiewicz M. 1996. Morphometrical characteristic of the common shrew (*Sorex araneus* L.) from interracial hybrid zones // *Hereditas*. 125: 201-207.

Corbet G.B. 1978. The mammals of Palaearctic region: a taxonomic review. London and Ithaca. 314 p.

Creste S., Neto A., Figueira A. 2001. Detection of single repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining // *Plant Mol. Biol. Reporter.* 19: 299-306.

Dolgov V.A. 1968. The peculiarities and variability of odontological features of Palearctic shrews (Mammalia, *Sorex*) // *Tr. Zool. Muz. MGU.* 10: 179-199. (In Russian).

Dolgov V.A. 1985. The shrews of the Old World. Moscow. 221 p. (In Russian).

Felsenstein J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. *Distributed by the author.* Washington, Seattle. (http://evolution.genetics.washington.edu/phylip.html, on March 24, 2008).

Hausser J. 1984. Genetic drift and selection; their respective and weights in the morphological and genetical differentiation of four species of shrews in Southern Europe (Insectivora, Soricidae) // *Z. Zool. Syst. Evolutionsforsch.* 22: 302-320.

Hausser J., Bosshard F., Taberlet P., Wójcik J. 1991. Relationships between chromosome races and species of Sorex of the araneus group in the western Alps // *Mém. Soc. Vaud. Sci. Natur.* 19: 79-95.

Král B., Radjabli S.I. 1974. Banding patterns and Robertsonian fusions in the western Siberian population of *Sorex araneus* (Insectivora, Soricidae) // *Folia Zool.* 23: 217-227.

Meyer A., Searle J.B. 1994. Morphological studies on British common shrews // *Folia Zool.* 43(1): 115.

Miller M.P. 1997. Tools for Population Genetic Analysis (TFPGA) version 1.3. A Windows program for the analysis of allozyme and molecular population genetic data. Northern Arizona University (http://www.marksgeneticsoftware.net/,on April 15, 2008).

Nei M. 1973. Analysis of gene diversity in subdivided populations // *Proc. Natl. Acad. Sci. USA.* 70: 3321-3323.

Nei M. 1975. Molecular population genetics and evolution. Amsterdam. 288 p.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals // *Genetics.* 89: 583-590.

Nei M., Li W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases // *Proc. Natl. Acad. Sci. USA.* 76: 5269–5273.

Nevo E., Ivanitskaya E., Beiles A. 2001. Adaptive radiation of blind subterranean mole rats: naming and revisiting the four sibling species of the *Spalax ehrenbergi* superspecies in Israel: *Spalax galili* (2n=52), *S. golani* (2n=54), *S. carmeli* (2n=58) and *S. judaei* (2n=60). Netherlands. 204 p.

Okulova N.M, Balakirev A.E., Orlov V.N. 2007. Craniometrical characteristic of some *Sorex araneus* chromosomal races // *Russian J. Theriol.* 6(1): 63-71.

Pavlinov I.Ya. 2004. Analysis of variation of the upper antemolars in the brown-toothed shrews (Mammalia: *Sorex*) by means of geometric morphometrics // *Zool. Zh.* 83(7): 869-875. (In Russian with English summary).

Polyakov A.V., Volobouev V.T., Borodin P.M., Searle J.B. 1996. Karyotypic races of the common shrew (*Sorex araneus*) with exceptionally large ranges: the Novosibirsk and Tomsk races of Siberia // *Hereditas*. 125: 109-115.

Polyakov A.V., Zima J., Banaszek A., Searle J.B., Borodin P.M. 2000. New chromosome races of the common shrew *Sorex araneus* from Eastern Siberia // *Acta Theriol.* 45(1): 11-18.

Polyakov A.V., Panov V.V., Ladygina T.Yu., Bochkarev M.N., Rodionova M.I., Borodin P.M. 2001. Chromosome evolution of the common shrew *Sorex araneus* L. in postglacial time on the south Ural and Siberia // *Russian J. Genet.* 37(4): 351-357.

Polyakov A.V., Onischenko S.S., Iliashenko V.B., Searle J.B., Borodin P.M. 2002. Morphometric difference between the Novosibirsk and Tomsk chromosome races of the common shrew (*Sorex araneus*) in a zone of parapatry // *Acta Theriol.* 47: 381-387.

Polyakov A.V., Volobouev V.T., Aniskin V.M. Zima J., Searle J.B., Borodin P.M. 2003. Altitudinal partitioning of two chromosome races of the common shrew (*Sorex araneus*) in the West Siberia // *Mammalia.* 67(2): 201-207.

Polyakov A.V., Beharav A., Krugman T., Avivi A., Nevo E. 2007. Ecological and phylogenetic significance of AFLP DNA diversity in 4 species of blind subterranean mole rats (*Spalax*) in Israel, (pp. 917-944) // Kelt D.A., Lessa E.P., Salazar-Bravo J., Patton J.L. (Eds). *Quintessential Naturalist: Honoring the Life and Legacy of Oliver P. Pearson. University of California Publications in Zoology.* Berkeley, Los Angeles, London. 981 p.

Raymond M.L., Rousset F. 1995. An exact test for population differentiation // *Evolution.* 49: 1280-1283.

Searle J.B. 1984. Three new karyotypic races of the common shrew *Sorex araneus* (Mammalia, Insectivora) and a phylogeny // *Syst. Zool.* 33: 184-189.

Searle J.B., Thorpe R. 1987. Morphometric variation of the common shrew (*Sorex araneus*) in Britain, in relation to karyotype and geography // *J. Zool.* 212: 373-377.

Searle J.B., Wójcik J.M. 1998. Chromosomal evolution: the case of *Sorex araneus*, (pp. 219-268) // Wójcik J.M., Wolsan M. (Eds.). *Evolution of Shrews.* Białowieża. 458 p.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting // *Nucleic Acids Res.* 23: 4407-4414.

Weir B.S. 1990. Genetic data analysis: methods for discrete population genetic data. Sunderland. 377 p.

Weir B.S., Cockerham C.C. 1984. Estimating *F*-statistics for the analysis of population structure // *Evolution.* 38(6): 1358-1370.

Wójcik J.M. 1993. Chromosome races of the common shrew *Sorex araneus* in Poland: a model of a karyotype evolution // *Acta Theriol.* 38: 315-338.

Yudin B.S. 1989. Insectivorous mammals of Siberia. Novosibirsk. 360 p. (In Russian).

Received July 1, 2009.
Accepted by N.Sh.Bulatova, November 17, 2009.
Published December 29, 2009.